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Infrageneric treatment of *Phalaris* (Canary grasses, Poaceae) based on molecular phylogenetics and floret structure

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Abstract. *Phalaris* L. (Poaceae, canary grasses) is a genus of 20 species found throughout the world with endemic, cosmopolitan, invasive and forage species. A variety of features in the genus underscore its importance for the study of polyploid evolution in relation to biodiversity, ecological niche expansion or contraction, endemism, and invasiveness. A formal and comprehensive infrageneric classification for *Phalaris* is lacking. This study utilises molecular phylogenetics (nuclear ITS and plastid *trn*T–F regions), morphological features (primarily floret structure) and chromosome cytology to present the first comprehensive taxonomic classification for the genus. Two subgenera (*Phalaris* and *Phalaroides*) and five sections (*Phalaris*, *Phalaroides*, *Caroliniana*, *Bulbophalaris*, *Heterachne*) are established here to accommodate the 20 *Phalaris* species. Keys to the subgenera and sections, morphological descriptions, and a list of synonymy are provided.

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Introduction

Recent molecular phylogenetic studies place *Phalaris* L. (Poaceae, grass family) in the Aveneae–Poeae complex of the subfamily Pooideae (Döring *et al*. 2007; Quintanar *et al*. 2007; Schneider *et al*. 2009; Saarela *et al*. 2015; Soreng *et al*. 2015). *Phalaris* comprises 20 species found throughout the temperate and neo-tropical regions of the world, with some being invasive or used as forage crops in many areas. The genus possesses a wide range of variation in ploidy levels, habitat, habit, floret structure (six floret types) and geographic ranges (Table 1; Voshell *et al*. 2011; Voshell and Hilu 2014). It includes endemics, such as *P. rotgesii* (Husnot) Baldini, *P. truncata* Guss. ex Bertol., *P. californica* Hook & Arn., *P. maderensis* (Menezes) Menezes and *P. lemmonii* Vasey, as well as the cosmopolitan *P. arundinacea* L. The latter has become a model species to study invasiveness (Galatowitsch *et al*. 1999; Lavergne and Molofsky 2004; Thomsen *et al*. 2012). Although polyploidy has played a major role in the evolutionary success of the Poaceae (Stebbins 1985; Hunziker and Stebbins 1987; Levy and Feldman 2002; Hilu 2006), its role in *Phalaris* is varied. Two basic chromosome numbers ($x = 6$ and $x = 7$) exist in *Phalaris*, pointing to aneuploidy in the genus (Baldini 1993, 1995). The $x = 6$ species, as well as the New World $x = 7$ species (except for *P. californica*), evolved through diploidy, whereas polyploidy played a role in the diversification of remaining Old World species with the emergence of tetraploid and hexaploid taxa (Voshell *et al*. 2011; Voshell and Hilu 2014). These attributes render *Phalaris* as an ideal model for experimental studies to evaluate some of the underlying biological factors that could affect biodiversity, such as endemism and invasiveness. Despite these intriguing biological features, an infrageneric taxonomic treatment for the genus is lacking. Current phylogenetic and phylogeographic studies of *Phalaris* (Voshell *et al*. 2011; Voshell and Hilu 2014) provide initial grounds for the assessment of species relationships and infrageneric delimitation when integrated with information from morphology, chromosome cytology and biogeography.

Taxonomic history

Phalaris has a rich taxonomic history dating back to the 1st Century common era (CE). The first historical record of the genus is a brief, 1st Century CE description by Dioscorides (most likely in reference to *P. canariensis* L.), which was accompanied by a Byzantine-era drawing (525 CE; Matthioli 1554). Anderson (1961) noted that the crude nature of the information and the accompanied sketch make it impossible to identify the plant with certainty. The following two species of *Phalaris* were named by Bauhin in 1623, before the advent of the binomial system: '*Phalaris major semine albo*' (*P. canariensis*) and '*Phalaris major semine nigro*' (cf. *P. minor* Retz.). Linnaeus (1753) included five species in the first edition of Species Plantarum, and added five more in later editions (Linnaeus 1755, 1763, 1767, 1771, 1781); some of these species were subsequently transferred to other genera (Baldini and Jarvis 1991; Baldini 1995). Lamarck (1778, 1783) and Lamarck and de Candolle (1805) circumscribed the genus to include 21 species; however, because of his broad definition of the genus, over half of the species were later re-assigned to other genera.

Table 1. Currently recognised *Phalaris* species and information regarding chromosome number and polyploid level, geographic range, habit and floret type (Baldini 1993, 1995)
A, annual; P, perennial

Species	Chromosome number	Range	Habit	Floret
Phalaris amethystina Trin.	$x = 7$; diploid	South America	A	3
Phalaris angusta Nees ex Trin.	$x = 7$; diploid	North and South America	A	3
Phalaris appendiculata Schult.	$x = 7$; diploid	Mediterranean andAfrica	A	6
Phalaris aquatica L.	$x = 7$; tetraploid	Mediterranean	P	5
Phalaris arundinacea L.	$x = 7$; tetraploid	Cosmopolitan	P	4
Phalaris brachystachys Link	$x = 6$; diploid	Mediterranean	A	2
Phalaris caesia Nees	$x = 7$; hexaploid	Mediterranean and Africa	P	4
Phalaris californica Hook & Arn.	$x = 7$; tetraploid	North America	P	3
Phalaris canariensis L.	$x = 6$; diploid	Mediterranean	A	1
Phalaris caroliniana Walt.	$x = 7$; diploid	North and South America	A	3
Phalaris coerulescens Desf.	$x = 7$; diploid	Mediterranean	P	6
Phalaris lemmonii Vasey	$x = 7$; diploid	North America	A	3
Phalaris lindigii Baldini	$x = 7$; unknown	South America	P	3
Phalaris maderensis (Menezes) Menezes	$x = 7$; tetraploid	Maderia, Portugal	A	5
Phalaris minor Retz.	$x = 7$; tetraploid	Mediterranean	A	5
Phalaris paradoxa L.	$x = 7$; diploid	Mediterranean	A	6
Phalaris peruviana H.Scholz & Gutte	$x = 7$; unknown	South America	P	3
Phalaris platensis Henrard ex Wacht.	$x = 7$; diploid	South America	A	3
Phalaris rotgesii (Husnot) Baldini	$x = 7$; diploid	Mediterranean	P	4
Phalaris truncata Guss. ex Bertol.	$x = 6$; diploid	Mediterranean	P	2

Trinius (1820) described nine species and was the first to use the sterile-floret features in the classification of the *Phalaris* species. His later revision (Trinius 1840) listed 15 species and included a taxonomic key. Steudel published taxonomic treatments in 1841 (Steudel 1841) and 1855 (Steudel 1855), in which he examined a large list of names associated with the genus, and assigned all but 25 as synonyms.

Although several regional treatments were subsequently produced, Anderson (1961) published the first worldwide revision since Steudel (1855); his treatment was based on morphological features and cytology and recognised 15 species. This treatment was followed by the comprehensive assessments of Baldini and Jarvis (1991) and Baldini (1993, 1995), where they recognised a total of 22 species including a synthetic octoploid developed as a forage crop in Australia. Baldini's (1995) treatment differed from the previous studies by recognising *P. appendiculata* Schult., *P. caesia* Nees, *P.* × *daviesii* S.T.Blake, *P. elongata* Braun-Blanq., *P. lindigii* Baldini, *P. peruviana* H.Scholz & Gutte and *P. rotgesii* (Husnot) Baldini. *Phalaris* × *daviesii* is an artificial octoploid hybrid derived from a cross between *P. minor* and *P. aquatica* L. (Blake 1956) and is used only as a forage plant in Australia.

None of these studies addressed the infrageneric groupings of the species. Tzvelev (1974, 1983) was the first to publish an infrageneric treatment of *Phalaris*, although it was regional, covering the taxa in the former Soviet Union. He recognised eight species in five sections (*Bulbophalaris* Tzvel., *Paraphalaris* Tzvel., *Heterachne* Dum. and *Phalaris*) and transferred *P. arundinacea* to *Phalaroides* Wolf. A recent molecular phylogenetic study showed that the entire genus, including *P. arundinacea*, is monophyletic (Voshell *et al*. 2011; Voshell and Hilu 2014) and questioned the circumscriptions of the sections recognised by Tzvelev. These phylogenetic studies demonstrated a split at the base of the tree into two lineages representing the $x = 6$ and $x = 7$ species. The latter lineage formed four clades with biogeographic affiliations and apomorphic floret structural features (Voshell *et al*. 2011; Voshell and Hilu 2014).

We present here the first comprehensive infrageneric classification for *Phalaris* based on molecular phylogenetics, reproductive morphological features, cytology and biogeographic distribution. To establish a reliable infrageneric treatment, a robust phylogeny is essential. Therefore, we generated three separate datasets to conducted multiple, non-overlapping additional analyses to assess the individual effects on tree topology and support when we exclude (1) accessions or individual with missing data, (2) accessions from species with polyploid genomes and (3) accessions resulting in sequence duplications. We also evaluated the topological incongruence between the plastid-based and the nuclear-based phylogenetic trees that appeared in the study of Voshell *et al*. (2011). On the basis of the nuclear biparerntal ITS data, *P. californica* emerged as sister to the New World clade, whereas it appeared sister to all other members of the genus using the maternal *trn*T–F dataset (Voshell *et al*. 2011).

Materials and methods

Morphological features and scanning electron microscopy

The floret structure appears to follow a trend when mapped on the phylogenetic tree and, thus, may represent useful synapomorphies in the infrageneric classification. These features include floret shape, sterile lemma structure and number, and glume structure. Information on floret morphology was obtained from literature (Anderson 1961; Baldini 1993, 1995). To better document these features, we provide here new scanning electron

microscope (SEM) images for *Phalaris* florets (Fig. 1). To generate the SEM pictures, single florets were taken from dried specimens, mounted on standard specimen stubs with double-sided adhesive tape and coated with carbon and gold, before observation with a Philips 515 (Germany), 15.2-kV scanning microscope following Baldini (1993).

Chromosome study

Chromosome number and morphology were observed for *P. angusta*, *P. arundinacea* and *P. californica* (Appendix 1) by using acetocarmine staining. Root tips from adult plants grown in the greenhouse were harvested and kept at 4°C overnight to shorten the chromosomes and halt the cell cycle at metaphase. Root tips were fixed in Carnoy's solution (3 : 1, ethanol : acetic acid) and stored at 4°C for analysis. The root tips were then placed in 1 : 1 ethanol : HCl maceration solution for 3 min, soaked in a 45% acetic acid solution containing 1% carmine for 2–3 min before being squashed on a slide under a coverslip. The slides were heated with an ethanol flame and pressed flat. Permanent slides were made by removing the cover with liquid nitrogen, dehydrating the material in ethanol and mounting a coverslip with clear nail varnish. Chromosomes were viewed using an Olympus CH-2 light microscope (USA) and photographed under the 100× objective with an iPod touch camera.

Molecular phylogenetics

For the molecular phylogenetic analysis, a dataset was constructed by combining nuclear ITS and plastid *trn*T–F sequences generated in Voshell *et al*. (2011) and Voshell and Hilu (2014) as described in Voshell *et al*. (2011). The concatenated ITS and *trn*T–F dataset differs from those in our previous studies because of the following: (1) we excluded *P. peruviana* because of the excessive amount of missing data and the low quality of the sequences; *Phalaris peruviana*

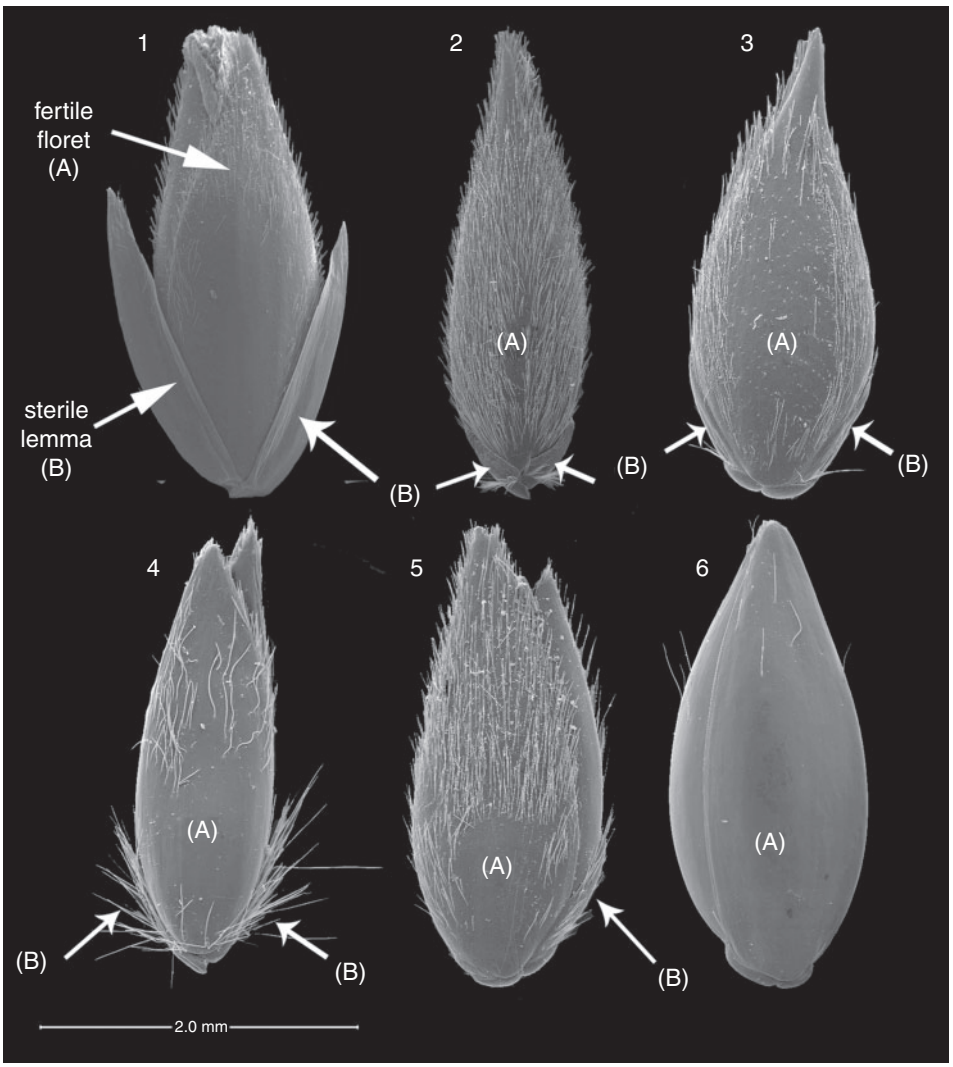

Fig. 1. Scanning electron microscope images of the six floret types recognised in *Phalaris*. The fertile floret (A) and sterile lemmas (B) are labelled. *Phalaris* florets range from 2 to 6 mm in length. *P. canariensis* (1), *P. truncata* (2), *P. platensis* (3), *P. arundinacea* (4), *P. minor* (5) and *P. paradoxa* (6).

is known only from the type specimen and the leaf sample received was in poor condition; (2) we eliminated accessions with identical sequences, a step that resulted in improved resolution and support in the reconstructed tree; and (3) we excluded the polyploid taxa sequences to generate a dataset that contained only the diploid species to explore the effect of polyploidy on phylogenetic reconstruction. Soltis *et al*. (2008) showed that the inclusion of polyploid species with diploids affects tree topology and support. We compare the topology of trees generated from these datasets with those obtained from the master dataset.

The ingroup dataset in the present study comprised 18 species. *Anthoxanthum monticola* (Bigelow) Veldkamp and *Hierochloe equiseta* Zotov were used as outgroup species because of their close phylogenetic proximity to *Phalaris* (Döring *et al*. 2007; Quintanar *et al*. 2007; Schneider *et al*. 2009; Saarela *et al*. 2015) and prior documented effectiveness in *Phalaris* tree reconstruction (Voshell *et al*. 2011; Voshell and Hilu 2014). Sequences were manually aligned in Quickalign ver. 1.6.0 (http://bioinfweb.info/Software/QuickAlign, accessed 2008). The combined alignment of the ITS and *trn*T–F sequences was 2127 nucleotides in length. A poly C region in the *trn*L–F region as well as a conserved region towards the 3′-end of the *trn*T–L region were excluded because they contained an excessive amount of missing data. The GenBank accessions used in the phylogenetic analysis here are noted in Appendix 1.

The combined dataset was analysed using Bayesian inference (Drummond and Rambaut 2007) and RAxML (Stamatakis 2014). The aligned sequences were analysed in jModelTest ver. 2.1.1 (Darriba *et al*. 2012) to select a suitable substitution model, and the GTR+G+I model was chosen on the basis of the Akaike information criterion (AIC). For the Bayesian inference analysis, the data files were prepared using BEAUTI ver. 1.6.2 (Drummond and Rambaut 2007) and the analyses were conducted in BEAST ver. 1.6.2 (Drummond and Rambaut 2007). The estimated sample size (ESS) was checked using Tracer ver. 1.5 (A. Rambaut and A. J. Drummond, see http://tree.bio.ed.ac.uk/software/tracer, accessed 2012) and the plotted posterior probability (PP) estimates for all runs were visually inspected to check for convergence. The analysis was run for 10 million generations by using the GTR+G+I substitution model and four gamma rate categories. All other parameters were left at the default settings and a 10% burn-in was used. BEAST was allowed to select the outgroup species and, consequently, the tree was rooted with *Hierochloe equiseta* and *Anthoxanthum monticola*, as expected.

A maximum likelihood analysis was conducted through the CIPRES portal using RAxML ver. 8 (Stamatakis 2014). *Anthoxanthum monticola* and *Hierochloe equiseta* were selected as outgroup taxa and 1000 replicates were run using the default settings and GTG+G+I model. PAUP* ver. 4.0b (Swofford, see http://paup.csit.fsu.edu, accessed 2008) was used to convert the data into tree files with support values, which were viewed in FigTree ver. 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/, accessed 2008). All trees were visualised and prepared for publication using FigTree ver. 1.3.1.

Results

Evidence from molecular phylogeny

In both the Bayesian and RAxML analyses of the master dataset (includes both diploids and polyploids), the phylogenetic trees based on the concatenated ITS and *trn*T–F datasets (Figs 2, 3) showed maximum support for the monophyly of *Phalaris* and a split at the base of the tree into two clades representing the $x = 6$ and the $x = 7$ species (Figs 2, 3; 1.00 PP and 0.98 PP; BS (bootstrap) 100%, BS <50% respectively, for $x = 6$ and $x = 7$ clades). In both analyses, the $x = 7$ lineage split into an Old World clade (OW, 100% BS, 1.00 PP) and a lineage (0.98 PP; BS <50%) containing two clades, one comprising the New World species (NW, 97% BS, 1.00 PP) and the other encompassing

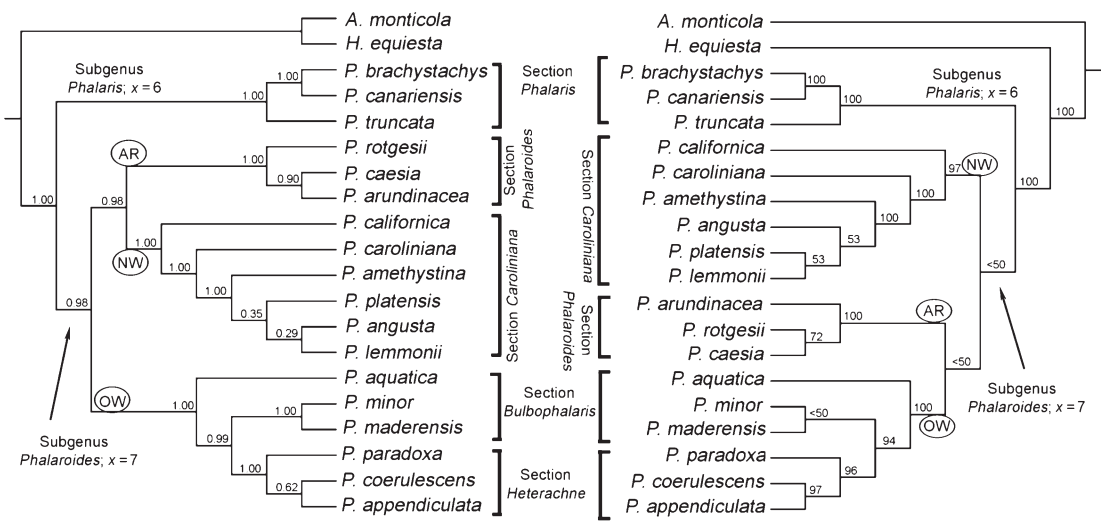

Fig. 2. Phylogenetic reconstruction of *Phalaris* on the basis of combined ITS and *trn*T–F regions using Bayesian inference (left) and RAxML (right), with respective posterior probabilities and bootstrap values noted. The proposed subgenera and sections are identified and the basic chromosome numbers for the two major clades (subgenera) are cited. Old World clade (OW), New World clade (NW), and Arundinacea clade (AR).

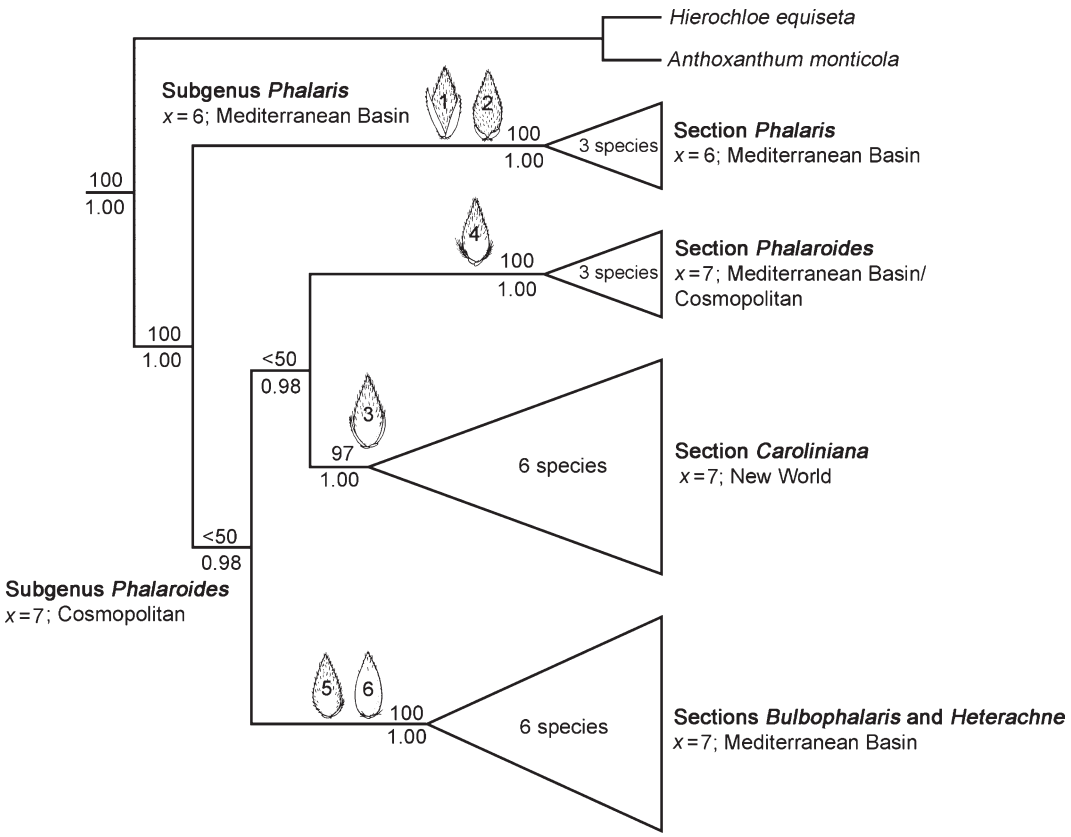

Fig. 3. Bayesian phylogenetic reconstruction of *Phalaris* on the basis of combined ITS and *trn*T–F regions, illustrating infrageneric classification, geographic affinities and associated floret synapomorphies.

P. rotgesii–P. arundinacea–P. caesia that received maximum support, and which we will refer to at this point as the Arundinacea clade (AR). It is to be noted that a strain of *P. arundinacea* native to North America has been reported (Merigliano and Lesica 1998); however, the species has a centre of origin in the Mediterranean region (Voshell and Hilu 2014).

The Bayesian and RAxML trees differed topologically in only two cases, but the incongruences lacked support and have no effect on the proposed classification of *Phalaris* because the major clades are resolved and well supported. The RAxML and Bayesian phylogenetic trees differed in the placement of the Arundinacea clade where it emerged sister to the clade containing the remaining Old World species in the former, but sister to the New World species in the latter. We chose to rely on the Bayesian tree topology because it received higher support than did the RAxML tree (<50 BS, 0.98 PP; Fig. 2). The second incongruence concerned the placement of *P. platensis* Henrard ex Wacht. within the New World clade. *Phalaris platensis* appears as sister to *P. lemmonii* in the RAxML tree instead of being sister to *P. angusta*. Support was lacking or extremely low in both cases.

In previous analyses that included *P. peruviana* in the dataset, this species unexpectedly (discussed later) emerged sister to all *Phalaris* species (Voshell *et al*. 2011). Therefore, we opted to re-analyse the dataset without this species. The exclusion of *P. peruviana* resulted in a tree (Figs 2, 3) topologically similar to that obtained in Voshell *et al*. (2011). The exclusion of duplicate samples did not lead to topological changes, but appeared to have a positive effect on the tree by increasing support at some nodes (Figs 2, 3). Analysis of the diploid-only dataset resulted in an overall topology similar to that of the tree generated from using both diploid and polyploid species.

Evidence from cytology

Chromosome morphology is used here to address questions relating to the placement of *P. californica* in the maternal *trn*T–F tree as sister to the remaining *Phalaris* species (Voshell *et al*. 2011) and the emergence of *P. arundinacea* as sister to the New World clade. The karyotype for *P. arundinacea* contains four pairs of large metacentric chromosomes (Fig. 4A, Chromosomes 1–4). No secondary constrictions were detected. The remaining 10 pairs of chromosomes were smaller and of similar size. Six of those pairs were metacentric to submetacentric, whereas the other four pairs were subtelocentric to acrocentric. The karyotype of *P. angusta*, an example of a New World species, is distinct from those of the Old World species that contain secondary constriction sites and many metacentric chromosomes (G. Winterfeld, S. M. Voshell, H. Becher, K. W. Hilu and M. Röser, unpubl. data). No secondary constriction sites were observed in *P. angusta* (Fig. 4C, D). The longest chromosomes were one pair of metacentric and one pair of submetacentric chromosomes

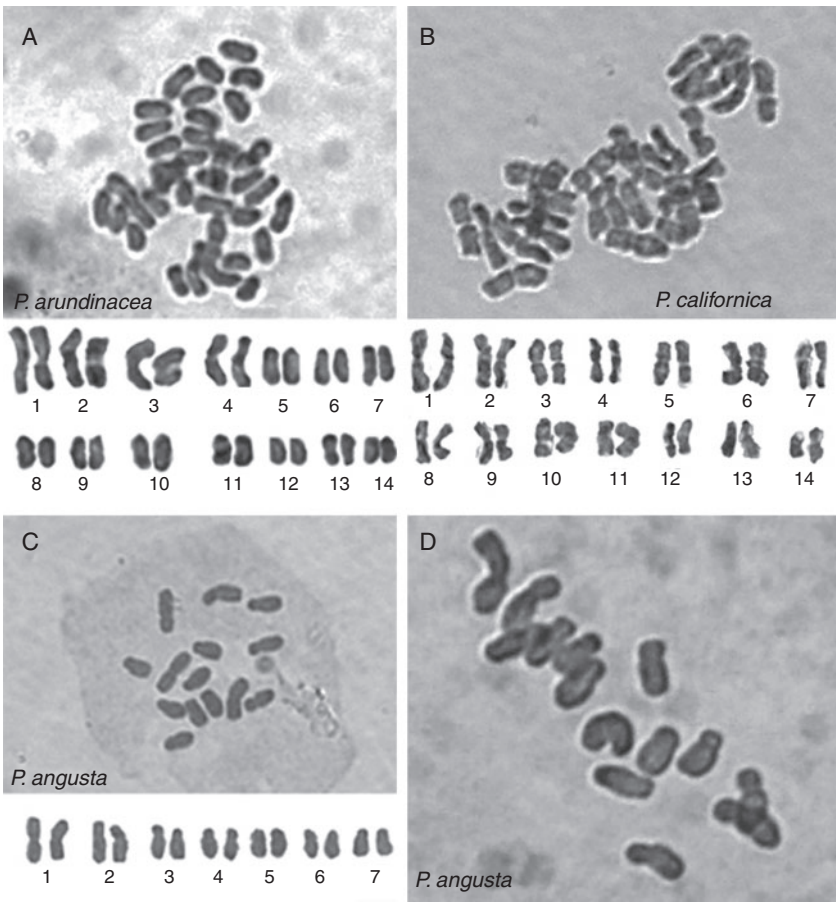

Fig. 4. Acetocarmine-stained metaphase spreads and karyotypes for two New World *Phalaris* species and *P. arundinacea*. A. *P. arundinacea*; $2n = 28$. Examples of chromosome morphology are: Chromosome 1, metacentric; Chromosome 5, acrocentric. B. *P. californica*; $2n = 28$. Chromosome 1, metacentric; Chromosome 7, submetacentric. C, D. *P angusta*; $2n = 14$. Chromosome 1, metacentric; Chromosome 2, submetacentric; Chromosome 3, acrocentric.

(Fig. 4C, Chromosomes 1 and 2 respectively). The remaining five pairs of chromosomes were small, acrocentric and of similar length. The karyotype for *P. californica* lacks the secondary constriction sites characteristic of the Old World species. It contains mostly metacentric and submetacentric chromosomes, and possesses acrocentric chromosomes similar to those seen in *P. angusta* and *P. arundinacea* (Fig. 4). The *P. californica* karyotype does not completely match those of the New World or Arundinacea species in that it contains fewer acrocentric chromosomes.

Evidence from morphology

The floral structure of *Phalaris* offers useful features for the taxonomic classification of the species (Fig. 1). The spikelets consist of two glumes of similar size displaying variation in shape, size, vestiture and presence or absence of a keel or a wing (Fig. 1). The glumes enclose a single fertile floret subtended by one or two highly reduced sterile ones (Fig. 1). In two rare cases, two fertile florets per spikelet were found in isolated populations of *P. aquatica* and *P. caesia* (Baldini 1993). SEM images of the six floret types are illustrated in Fig. 1 and their parts are labelled.

Discussion

Comparative phylogenetics of Phalaris

To affirm the phylogenetic structure of *Phalaris* and use it as a platform for an infrageneric taxonomic treatment, we (1) carried out RAxML analyses because topological differences sometimes are notable when compared with the Bayesian inference, (2) excluded *P. peruviana* that had a questionable phylogenetic position, (3) excluded duplicate samples and (4) removed sequences of polyploid species to assess their effect on the phylogeny of the genus. In the case of *Phalaris*, the RAxML analyses revealed some incongruences with the Bayesian inference, although the differences received low support and were inconsequential to the overall topology and the subgeneric and sectional classification of the genus (Figs 2, 3). In a previous study that included *P. peruviana*, the emergence of this species as the sister to the remainder of the *Phalaris* taxa (Voshell *et al*. 2011) raised questions because it is endemic to Peru and morphologically resembles the other New World species with Type 2 florets. We believe that the reason for this phylogenetic placement of *P. peruviana* is the low quality of the sequence data that resulted in partial sequences. The genomic

DNA was isolated from leaf material of an old, single voucher available for this species. Consequently, the sequences for this species were short and ambiguous. The congruence between the trees on the basis of the inclusion and exclusion of *P. peruviana* (Figs 2, 3; and Voshell *et al*. 2011) demonstrated that the exclusion of this taxon does not affect the overall structure of *Phalaris* phylogeny.

Our exclusion of duplicate accessions did not affect the topology, but appeared to have a positive effect on the tree by increasing support for some nodes (Figs 2, 3). Analysis of the diploid-only dataset resulted in a topology overall similar to that of the tree generated from using a combined diploid and polyploid dataset. Therefore, the presence of sequences from polyploid taxa does not have a negative effect on phylogenetic reconstruction in *Phalaris*, as has been noted in other studies (Soltis *et al*. 2008).

Floret evolution in Phalaris

The key morphological features responsible for the placement of *Phalaris* in the 'mostly Aveneae' lineage *sensu* Döring *et al*. (2007) and Poeae 'Chloroplast group 1 – Aveneae type' *sensu* Soreng *et al*. (2015) are the reduced floral elements and the arrangement of the sterile and fertile components of the spikelet. *Phalaris* appears in isolated positions in these molecular phylogenetic treatments (Döring *et al*. 2007; Quintanar *et al*. 2007).

Despite reduction in floral structure in *Phalaris*, six floret morphotypes are recognised (Anderson 1961; Baldini 1993, 1995; Voshell *et al*. 2011); these characters can be mapped onto the clades resolved in the phylogenetic analyses as potential synapomorphies, and subsequently, in the infrageneric classification (Figs 1, 3). Species in the $x = 6$ clade (*P. canariensis, P. brachystachys* Link, *P. truncata*) have very distinctive wide, chaffy bract-like sterile lemmas (Types 1, 2; Fig. 1). In contrast, the sterile lemmas in the $x = 7$ species range from thin and hairy to completely lacking (Types 3–6; Fig. 1). Within the $x = 7$ lineage, species of the New World clade (*P. californica, P. angusta* Nees ex Trin., *P. caroliniana* Walt., *P. platensis*, *P. amethystina* Trin., *P. lemmonii*) and the Arundinacea clade (*P. arundinacea*, *P. caesia*, *P. rotgesii*) display two thin sterile lemmas with varied amounts of pubescence (Types 3, 4; Fig. 1). The Old World species (*P. paradoxa* L., *P. coerulescens* Desf., *P. minor*, *P. aquatica*, *P. maderensis*) possess either one thin sterile lemma or lack both (Types 5, 6; Fig. 1). Therefore, the $x = 6$ lineage possesses the largest and most prominent sterile lemmas. Within the $x = 7$ clade, the Arundinacea species have sterile lemmas that are reduced to hairy tufts, whereas the New World species have long, thin sterile lemmas with less hair. The Old World species show the greatest degree of reduction in the genus, with florets possessing either one sterile lemma or none at all.

The structure of the florets appears to have an association with biogeography. Voshell and Hilu (2014) conducted dispersal–vicariance analyses to discern geographic affinities for major clades. The study showed the Mediterranean region as the area of the origin of *Phalaris*, and the centre of diversity for the $x = 6$ and the Old World $x = 7$ species. The study also suggested that a diploid ancestor of *P. arundinacea* migrated from the Mediterranean Basin to North America (Voshell and Hilu 2014) over the Bering Land Bridge (~9–5 million years ago) into western North America, with subsequent dispersal and speciation to the rest of the New World. The small floret size and protruding sterile lemmas associated with the Arundinacea and the New World species could have been instrumental in their dispersal by animals and for the subsequent speciation (Cheplick 1998; Cousens *et al*. 2008). In contrast, clades with species having larger florets exhibit limited geographic distribution.

Karyotypes of New World species

Comparison of acetocarmine-stained chromosomes from New World *P. angusta* and *P. californica* with Old World *P. arundinacea* showed evidence of shared ancestry. The karyotype of the New World species *P. angusta* displayed five small acrocentric chromosomes, a feature not found in the Old World diploids (G. Winterfeld, S. M. Voshell, H. Becher, K. W. Hilu and M. Röser, unpubl. data). The karyotype of *P. arundinacea* does contain medium-sized metacentric chromosomes and small acrocentric chromosomes, indicating potentially shared ancestry with *P. angusta* (Fig. 4). Although *P. arundinacea* is tetraploid, its closest relative and potentially putative ancestor, *P. rotgessii*, is diploid. Voshell and Hilu (2014) suggested that it is likely that a diploid progenitor of *P. arundinacea* travelled across the Bering Land Bridge, giving rise to the New World species. These karyotype results demonstrated close evolutionary relationship between the two respective clades, as shown in the phylogenetic tree (Figs 2, 3).

Phylogenetic reconstruction of the genus places *P. californica* as sister to the New World clade in the bipaternal ITS tree and as sister to remaining *Phalaris* species in the maternal *trn*T–F tree (Voshell *et al*. 2011). Morphologically, *P. californica* is similar to the other New World species in having a Type 3 floret with long, narrow sterile lemmas, and it is found in the same geographic range as is *P. angusta* (Voshell *et al*. 2011). Unlike all other New World species, *P. californica* is a tetraploid. Voshell *et al*. (2011) and Voshell and Hilu (2014) found that in the maternal *trn*T–F dataset *P. californica* shares unique indels and mutations with the outgroup species. These observations may account for its phylogenetic placement in the maternal tree, and supports the hypothesis that *P. californica* may be an intergeneric hybrid. This hypothesis needs to be tested experimentally to assess its accuracy and to identify the putative parents. The karyotype of *P. californica* also displays some similarities to that of *P. arundinacea*, primarily the large to medium metacentric chromosomes; it also shares a few acrocentric chromosomes with *P. angusta*, but not the 10 pairs one would expect of an autotetraploid (Fig. 4). These karyotypes provide further support for the intergeneric hybrid origin of *P. californica*. Regardless of the origin of *P. californica*, the cytological, geographic and phylogenetic data point to the taxonomic affinity of the species to those in the New World clade (Fig. 2).

Assessments of notable species-level treatments

Among the three $x = 6$ species (Table 1), *P. brachystachys* has been included under *P. canariensis* (Baldini 1995). However, *P. brachystachys* differs morphologically from *P. canariensis* in

having short and wide sterile lemmas and seeds that shatter at maturity, compared with the distinctively long and narrow sterile lemmas and seeds that remain in the spikelet in *P. canariensis* (Baldini 1995). In addition, morphological intermediates between the two species have not been reported, implying lack of gene flow. We, thus, treat the two as distinct species. In the $x = 7$ lineage, *P. arundinacea*, *P. rotgesii* and *P. caesia* are sometimes lumped under *P. arundinacea* (Baldini 1993). These three taxa also differ in size and hairiness of the floret. The species are reproductively isolated by polyploidy and, thus, should be consistently treated at the species level as proposed in Baldini (1995).

Phalaris paradoxa and *P. appendiculata* are morphologically the most variable species in the genus. *Phalaris appendiculata* was either included with *P. paradoxa* as a subspecies (Baldini 1993, 1995) or treated as a distinct species. *Phalaris appendiculata* can be distinguished from *P. paradoxa* by the unique presence of complex clusters of single-floret fertile spikelets surrounded by numerous sterile spikelets. Phylogenetic analyses have revealed that the species are closely related (Voshell *et al*. 2011; Voshell and Hilu 2014; the present study). Field and herbarium studies have pointed to some morphological intermediates between the two (Baldini 1993, 1995); thus, it is likely that a limited amount of gene flow still occurs among their populations in areas of geographic overlap (Baldini 1995). *Phalaris paradoxa* has a broader range than has *P. appendiculata*; the latter is confined to northern Africa and Ethiopia (Baldini 1995). Thus, *P. appendiculata* should be treated as a distinct species on the basis of pronounced and unique reproductive morphology, geographic range and the presence of molecular markers (substitutions and indels) not shared with *P. paradoxa* (Voshell *et al*. 2011).

Phalaris lindigii is endemic to South America (Baldini 1995). Recent studies (Voshell *et al*. 2011; Voshell and Hilu 2014) have demonstrated the potential hybrid origin of this species from *P. arundinacea* and *P. aquatica*, which are two species that have been introduced to the region by human activities. *Phalaris lindigii* emerges sister to *P. arundinacea* in the maternal *trn*T–F tree and to *P. aquatica* in a bipaternal ITS tree (Voshell and Hilu 2014). Despite the potential hybrid origin of *P. aquatica*, the species is morphologically distinct in spikelet structure (Baldini 1995; Voshell *et al*. 2011) and it will be treated at the species level at this point (Baldini 1995).

The remaining species possess distinct morphologies and, thus, their taxonomic status has not been disputed (Anderson 1961; Baldini 1993, 1995). Consequently, we recognise 20 species in *Phalaris*.

Taxonomic treatment

Considering the total evidence, the trees obtained with the combined ITS and *trn*T–F dataset show two distinct lineages with characteristic floret morphologies (Types 1 and 2 *v.* Types 4–6; Figs 1, 3) and aneuploid cytotypes ($x = 6$ *v.* $x = 7$). The three strongly supported clades in the $x = 7$ lineage (Figs 2, 3) are each associated with distinct floret types, geographic affinities (Voshell and Hilu 2014) and chromosome number and morphology. These attributes provide a reliable basis for the *Phalaris* infrageneric classification proposed below. We propose two subgenera, representing the two major lineages corresponding to the $x = 6$ and $x = 7$ cytotypes. Within the $x = 7$ lineage, we recognise the three major clades at the sectional level. Brief descriptions for all these taxa are provided, as well as the type species, synonyms and species. This information is mainly based on earlier treatments by Baldini and Jarvis (1991) and Baldini (1993, 1995).

Subgeneric classification

Phalaris L., *Sp. Pl.* 1: 54 (1753)

Type: *Phalaris canariensis* L.

Subgenus ***Phalaris***

Description

Sterile lemmas chafflike, 2–3 mm long and narrow or 1/5–1/10 the length of the fertile floret and wide.

Section ***Phalaris***

Phalaris sect. *Euphalaris* Asch. & Graebn., *Syn. Mitteleur. Fl.* 2(1): 15 (1898), *nom. inval., p.p.*

Description

Perennial rhizomatous with swelling base-stem, or annual herbs; ligule acuminate, cylindrical, often lacerate, 3–7 mm long; panicle 2–8 cm long, cylindrical, ovate to subovoid; glumes 6–10 mm long, winged; sterile lemmas chafflike, 2–3 mm long; fertile lemmas equal to subequal 4–6 mm long. Chromosome basic number: $x = 6$.

Phalaris canariensis L., *Sp. Pl.* 54 (1753)

Phalaris ovata Moench, *Methodus* 208 (1794), *nom. illeg., nom. superfl.*; *Phalaris avicularis* Salisb., *Prodr. Stirp. Chap. Allerton* 17 (1796), *nom. illeg., nom. superfl.*; *Phalaris canariensis* subsp. *typica* Posp., *Fl. Oesterr. Küstenl*. 1: 59 (1897), *nom. inval.*

Phalaris canariensis var. *debilis* Tocl & Rohlena, *Sitzungsber. Königl. Böhm. Ges. Wiss., Math.-Naturwiss. Cl*. 49: 1 (1902).

Phalaris canariensis var. *subcylindrica* Thell., *Vierteljahrsschr. Naturf. Ges. Zürich* 56: 271 (1912).

Phalaris canariensis f. *vivipara* Junge, *Jahrb. Hamburg. Wiss. Anst*. 30: 123 (1912).

Phalaris canariensis f. *bracteata* Jansen & Wacht., *Ned. Kruidk. Arch*. 6: 135 (1917).

Phalaris canariensis L. var. *villosula* Jansen & Wacht. *Ned. Kruidk. Arch*. 52: 213 (1942).

Phalaris canariensis var. *tenuis* Jansen & Wacht., *Ned. Kruidk. Arch*. 52: 213 1942).

Phalaris canariensis f. *colorata* Jansen & Wacht., *Ned. Kruidk. Arch*. 52: 213 (1942).

Phalaris brachystachys Link, *Neues J. Bot.* 1(3): 134 (1806)

Phalaris canariensis var. *brachystachys* (Link) Posp., *Fl. Oesterr. Küstenl*. 1: 59 (1897); *Phalaris brachystachys* var. *typica* Paunero, *Anales Jard. Bot. Madrid* 8: 492 (1948), *nom. inval.*

Phalaris quadrivalvis Lag., *Gen. Sp. Pl.* 3 (1816).

Phalaris nitida C.Presl, *Cyper. Gramin. Sicul*. 26 (1820).

Phalaris brachystachys var. *robusta* Thell., *Mém. Soc. Sci. Nat. Math. Cherbourg* 38: 88 (1912).

Phalaris truncata Guss ex. Bertol., *Fl. Ital.* 2: 777 (1835)

Phalaris brachystachys var. *truncata* (Guss.) Paunero, *Anales Jard. Bot. Madrid* 8: 492 (1948); *Phalaris truncata* var. *typica* Maire & Weiller in R.Maire, *Fl. Afr. Nord* 2: 20 (1953), *nom. inval.*

Phalaris truncata f. *angustata* Trab., in J.A.Battandier & L.C.Trabut, *Fl. Algérie* 140 (1895); *Phalaris truncata* var. *angustata* (Trab.) Maire & Weiller in R.Maire, *Fl. Afr. Nord* 2: 20 (1953).

Phalaris truncata var. *villiglumis* Maire, *Fl. Afr. Nord* 2: 20 (1953), *nom. inval.*

[*Phalaris aquatica auctt. non* L., *p.p.*].

Subgenus ***Phalaroides*** (Wolf) Voshell, Baldini & Hilu, *comb. et stat. nov.*

Phalaroides Wolf, *Gen. Pl.* 11 (1776).

Type: *Phalaris arundinacea* L.

Typhoides Moench, *Methodus* 1: 201 (1794), *nom. illeg., nom. superfl.*

Baldingera P. Gaertn., B.Meyer & Scherb., *Oekon. Fl. Wetterau* 1: 96 (1799), *nom. illeg., nom. superfl.*

Digraphis Trin., *Fund. Agrost*. 127 (1822), *nom. illeg., nom. superfl.*

Endallex Raf., *Bull. Bot.* (Geneva) 1: 220 (1830), *nom. illeg., nom. superfl.*

Phalaridantha St.-Lag., in A.Cariot, *Etude Fl*. 8th edn, 2: 900 (1889), *nom. illeg., nom. superfl.*

Description

Sterile lemmas hairy or tufts of hair, 1 or 2, equal or inequal, 0.8–3.5 mm long, if glabrous one or both sterile lemmas reduced.

Section ***Phalaroides***

Description

Perennial, rhizomatous herbs; ligule subtruncate, truncate and spathulate, 7–15 mm long; panicle 5–30 cm long, compact, lobate, to branched; glumes 2–7 mm long, 3 nerved, not winged to narrow winged; sterile lemmas 2, equal, 1–2.5(3) mm long, feathery; fertile lemmas 2–5 mm long, scarcely feathery to sparsely pubescent. Chromosome basic number: $2x = 7$.

Phalaris arundinacea L., *Sp. Pl.* 55 (1753)

Typhoides arundinacea (L.) Moench, *Methodus* 202 (1794); *Digraphis arundinacea* (L.) Trin., *Fund. agrost*. 127 (1822); *Baldingera arundinacea* (L.) Dumort., *Observ. Gramin. Belg*. 130 (1823); *Endallex arundinacea* (L.) Raf., *Bull. Bot.* (Geneva) 1: 220 (1830); *Phalaridantha arundinacea* (L.) St. Lag., in A.Cariot, *Etude Fl*., 8th edn, 2: 900 (1889); *Phalaris arundinacea* var. *genuina* Hack., *Bull. Herb. Boissier* 9: 646 (1899), *nom. inval.*; *Phalaris arundinacea* subsp. *typica* Paunero, *Anales Jard. Bot. Madrid* 8: 489 (1948), *nom. inval.*; *Phalaroides arundinacea* (L.) Rauschert, *Feddes Repert*. 79(6): 409 (1969).

Arundo colorata Aiton, *Hort. Kew*. 1: 116 (1768); *Arundo riparia* Salisb., *Prodr. Stirp. Chap. Allerton* 24 (1796), *nom. illeg., nom. superfl.*; *Calamagrostis colorata* (Aiton) Sibth., *Fl. Oxon*. 37 (1794).

Phalaris arundinacea f. *ramosa* Gaudin, *Fl. Helv*. 1: 160 (1828).

Digraphis arundinacea f. *coarctata* Prahl, *Krit. Fl. Schlesw.-Holst*. 2(2) 245 (1890); *Phalaris arundinacea* f. *coarctata* (Prahl) Junge, *Jarbh. Hamburg. Wiss Anst. Beih.* 22: 60 (1905).

Phalaris arundinacea f. *ramifera* Junge, *Jarbh. Hamburg. Wiss Anst. Beih.* 22: 60 (1905).

Phalaris arundinacea f. *minor* Jansen & Wacht., *Nedl. Kruidk. Archief*. 6: 141 (1917).

[*Phalaris americana auctt. non* Elliott (American floras)].

Phalaris caesia Nees, *Fl. Afr. Austral.* 6 (1841)

Phalaroides arundinacea subsp. *caesia* (Nees) Tzvel., *Novosti Sist. Vyssh. Rast*. 10: 80 (1974); *Phalaroides caesia* (Nees) Holub, *Folia Geobot. Phytotax*. 12(4): 428 (1977).

Phalaris arundinacea var. *thyrsoidea* Willk., *Oesterr. Bot. Z*. 40(4): 145 (1890); *Phalaris arundinacea* f. *thyrsoidea* (Willk.) Paunero, *Anales Jard. Bot. Madrid* 8: 489 (1948).

Phalaris hispanica Coincy, *J. Bot. (Morot)* 8: 207 (1894); *Phalaris arundinacea* subsp. *hispanica* (Coincy) Kerguélen, *Bull. Soc. Bot. France* 123: 322 (1976); *Phalaroides hispanica* (Coincy) Holub, *Folia Geobot. Phytotax*. 12(4): 428 (1977).

Phalaris arundinacea subsp. *oehleri* Pilg., *Bot. Jahrb. Syst*. 43: 91 (1909); *Phalaroides arundinacea* subsp. *oehleri* (Pilg.) Valdés & H.Scholz, *Willdenowia* 36: 664 (2006).

Phalaris arundinacea var. *leioclada* Maire, *Bull. Soc. Hist. Nat. Afrique N*. 32: 217 (1941).

[*Phalaris arundinacea auctt. non* L. (African floras)].

Phalaris rotgesii (Foucad & Mandon ex Husn.) Baldini, *Webbia* 47(1): 13 (1993)

Baldingera arundinacea var. *rotgesii* Foucad & Mandon ex Husn., *Graminées* 87 (1899); *Baldingera arundinacea* form. stat. *rotgesii* (Foucad & Mandon ex Husnot) Foucaud & Mandon, *Bull. Soc. Bot. France 3 sér*., 47(7): 99 (1900); *Phalaris arundinacea* subvar. *rotgesii* (Foucad & Mandon ex Husnot) Fiori, *Fl. Italia* 4: 14 (1907); *Phalaris arundinacea* proles *rotgesii* (Foucad & Mandon ex Husnot) Litard., *Bull. Acad. Int. Geogr. Bot.* 100 (1909); *Phalaris arundinacea* var. *rotgesii* (Foucad & Mandon ex Husnot) Litard. ex Briq., *Prodr. Fl. Corse* 1: 71 (1910); *Phalaris arundinacea* race *rotgesii* (Foucad & Mandon ex Husnot) Jansen & Wacht., *Ned. Kruidk. Archief*. 142 (1917); *Thyphoides arundinacea* subsp. *rotgesii* (Foucad & Mandon ex Husnot) Gamisans, *Candollea* 29: 44 (1974); *Phalaroides rotgesii* (Foucad & Mandon ex Husnot) Holub, *Folia Geobot. Phytotax*. 12: 428 (1977); *Phalaroides arundinacea* subsp. *rotgesii* (Husnot) Valdés & H. Scholz, *Willdenowia* 36: 664 (2006).

Section ***Caroliniana*** Voshell, Baldini & Hilu, *sect. nov.*

Type: *Phalaris caroliniana* Walt.

Description

Perennial rhizomatous and annual herbs; ligule 3–8 mm long, truncate–subtruncate to lanceolate, sometimes lacerate; panicle 2–20 cm long, cylindrical, ovoid to subovoid, sometimes branched at the base; glumes 3.5–8.0 mm long,

keel winged to narrowly winged; sterile lemmas 2, equal, 0.8–3.5 mm long, scarcely feathery to densely pubescent; fertile lemmas 2.5–5.0 mm long, pubescent to scarcely pubescent, sometimes near the tip, or at the bottom. Chromosome basic number: $x = 7$.

Phalaris caroliniana Walt., *Fl. Carol.* 74 (1788)

Phalaris intermedia Bosc ex Poir., *Encycl., Suppl*. 1(1): 300 (1810).

Phalaris microstachya DC., *Cat. Pl. Hort. Monsp.* 131 (1813); *Phalaris intermedia* var. *microstachya* (DC.) Vasey, *Contr. USA Natl. Herb*. 3(1): 42 (1892).

Phalaris americana Elliott, *Sketch Bot. S. Carolina* 1(2): 101 (1817).

Phalaris occidentalis Nutt., *Trans. Amer. Philos. Soc., n.s*., 5: 144 (1837).

Phalaris trivilias Trin., (*Mém. Acad. Imp. Sci. Saint-Pétersbourg, Sér. 6, Sci. Math., Seconde Pt. Sci. Nat*. 5, 3(3): 55 (1839), *nom. inval., nom. nud*.

Phalaris angusta Nees ex Trin., *Sp. Gram.* 1, pl. 78 (1828)

Phalaris intermedia var. *angusta* (Nees ex Trin.) Chapm., *Fl. South USA* 569 (1865).

Phalaris angusta Nees in C.Martius, *Fl. Bras. Enum. Pl.* 2: 391 (1829), *nom. illeg., non* Nees ex Trin. (1828).

Phalaris chilensis J.Presl, *Reliq. Haenk*. 1: 245 (1830).

Phalaris intermedia var. *angustata* Beal, *Grasses N. Amer*. 2: 182 (1896).

Phalaris angusta f. *macra* Hack., *Anales Mus. Nac. Buenos Aires* ser. 3 24: 63 (1911).

Phalaris angusta f. *colorata* Jansen & Wacht., *Ned. Kruidk Archief*. 6: 139 (1917).

Phalaris angusta var. *robusta* Jansen & Wacht., *Ned. Kruidk Archief*. 6: 139 (1917).

Phalaris angusta f. *composita* Jansen & Wacht., *Ned. Kruidk Archief*. 6: 139 (1917).

Phalaris angusta f. *interrupta* Jansen & Wacht., *Ned. Kruidk Archief*. 6: 139 (1917).

Phalaris angusta f. *bracteata* Jansen & Wacht., *Ned. Kruidk Archief*. 6: 139 (1917).

[*Phalaris intermedia auctt. non* Bosc].

Phalaris amethystina Trin., *Mém. Acad. Imp. Sci. Saint-Pétersbourg, Sér. 6, Sci. Math., Seconde Pt. Sci. Nat.* 5, 3(3): 56 (1839)

Phalaris berteroniana Steud., *Syn. Pl. Glumac.* 1: 11 (1853).

Phalaris robinsoniana Nees ex Steud., *Syn. Pl. Glumac*. 1: 11 (1853).

Phalaris colchaguensis Phil., *Linnaea* 33: 276 (1864).

[*Phalaris angusta auct. non* Nees: C.B. von Trinius, *Linnaea* 10: 299 (1835)].

[*Phalaris microstachya auct. non* DC.: N.A.Desvaux, *Fl. Chilena* 6: 255 (1853)].

[*Phalaris intermedia auct. non* Bosc. ex Poir.: F.R.A.Johow, *Estud H. Juan Fernandez* 134 (1896)].

Phalaris lemmonii Vasey, *Contr. USA Natl. Herb.* 3: 42 (1892)

Phalaris platensis Henrard ex Wacht. in H. Heukels, *Schoolfl. Nederl.* 843 (1934)

Phalaris arechavaletae Herter, *Revista Sudamer. Bot*. 9: 105 (1953), *nom. illeg., nom. superfl.*

Phalaris intermedia f. *platensis* Arechav., *Anales Mus. Nac. Montevideo* 1(4): 298 (1896); *Phalaris platensis* (Arechav.) Parodi ex Valencia, *Revista Argent. Agron*. 4: 298 (1937).

Phalaris californica Hook. & Arn., *Bot. Beechey Voy*. 1: 161 (1833)

[*Phalaris amethystina auct. non* Trin. (American floras)].

Phalaris peruviana H.Scholz & Gutte, *Willdenowia* 8: 379 (1978)

Phalaris lindigii Baldini, *Webbia* 49(2): 317 (1995)

Section ***Bulbophalaris*** Tzvel., *Novosti Sist. Vyssh. Rast.* 11: 70 (1974)

Phalaris sect. *Euphalaris* Asch. & Graebn., *Syn. Mitteleur. Fl.* 2(1): 15 (1898), *nom. inval., p.p.*

Type: *Phalaris tuberosa* L (=*P. aquatica* L.).

Description

Perennial rhizomatous often with swelling stem-base and annula herbs; ligule 3–8 mm long, cylindrical to subtruncate; panicle 3–15 cm long, cylindrical, ovato-lanceolate, ovato-spathulate; spikelets uniform, or in groups of 6 or 7, with 1 fertile floret surrounded at the bottom by (5)6–7 staminate florets (*P. appendiculata, P. coerulescens*) or 5 or 6 sterile spikelets (*P. paradoxa*); glumes 4–9 mm long, winged with margin entire or erose–dentate; sterile lemmas 1 or 2 inequal, 0.5–2 mm long, or both very reduced to obsolete (*P. appendiculata, P. paradoxa, P. coerulescens*); fertile lemmas 2.5–5 mm long. Chromosome basic number: $2x = 7$.

Phalaris minor Retz., *Observ. Bot.* 3 : 8 (1783)

Phalaris decumbens Moench, *Methodus* 208 (1794), *nom. illeg.*, *nom. superfl.*; *Phalaris arundinacea* var. *minor* (Retz.) Paunero, *Anales Jard. Bot. Madrid* 8: 489 (1948); *Phalaris minor* var. *genuina* Maire & Weiller in R.Maire, *Fl. Afr. Nord*. 2: 23. (1953) *nom. inval*.

Phalaris capensis Thunb., *Prodr. Pl. Cap*. 19 (1794).

Phalaris aquatica Thunb., *Prodr. Pl. Cap*. 19 (1794), *nom. illeg.*, *non* L. (1755).

Phalaris nepalensis Trin., *Sp. Gram*. 1(7), t.80 (1828); *Phalaris minor* var. *nepalensis* (Trin.) Bor, *Grass. Burma, Ceylon, India & Pakistan* 616 (1960).

Phalaris brevis Trin., *Mém. Acad. Imp. Sci. Saint-Pétersbourg, Sér. 6, Sci. Math., Seconde Pt. Sci. Nat.* 5, 3 (3): 50 (1839).

Phalaris ambigua Fig. & De Not., *Agrostgr. Aegypt* 10 (1853).

Phalaris gracilis Parl., *Pl. Nov*. 36 (1842); *Phalaris minor* var. *gracilis* (Parl.) Parl., *Fl. Ital.* 1: 70 (1848); *Phalaris minor* subsp. *gracilis* (Parl.) Arcang., *Comp. Fl. Ital*. 754 (1882); *Phalaris minor* f. *gracilis* (Parl.) Asch. & Graebn., *Syn. Mitteleur. Fl*. 2(1): 21 (1898).

Phalaris minor var. *integra* Trab., *Bull. Soc. Bot. France* 32(7): 394 (1885).

Phalaris minor var. *comosula* Heldr., *Bull. Herb. Boisser*. 4: 396 (1898).

Phalaris minor f. *haematites* Duval-Jouve & Paris ex Trab., in J.A. Battandier & L.C.Trabut, *Fl. Algérie* 141 (1895).

Phalaris minor f. *subcylindrica* Web. & Thell. ex Jansen & Wacht., *Ned. Kruidk. Archief.* 6: 131 (1917).

Phalaris minor f. *composita* Jansen & Wacht., *Ned. Kruidk. Archief*. 6: 132 (1917).

Phalaris minor f. *bracteata* Jansen & Wacht., *Ned. Kruidk. Archief*. 6: 132 (1917).

Phalaris minor f. *phaeosperma* Cavara, *Bull. Orto Bot. Regia Univ. Napoli* 9: 42 (1927).

Phalaris mauritii Sennen, *Diagn. Nouv*. 243 (1936).

Phalaris haematites var. *granulosa* Sennen & Mauricio, in Sennen, *Diagn. Nouv*. 244 (1936), *nom. inval*.

[*Phalaris aquatica auct. non* L.: C.L.Willdenow, *Sp. Pl*. 1: 326 (1797), *p.p.*].

Phalaris aquatica L., *Cent. Pl.* I 4 (1755)

Phalaris nodosa Murray, *Syst. Veg*., 13th edn, 88 (1774), *nom. illeg., nom. superfl.*

Phalaris tuberosa L., *Mant. Pl.* 557 (1771).

Phalaris commutata Roem. & Schult., *Syst. Veg*., 15th edn, 2: 403 (1817).

Phalaris altissima Menezes, *Cat. Phanerog. Madeira, Porto Santo* 58 (1894).

Phalaris tuberosa var. *alata* Trab., *Fl. Algérie* 141 (1895).

Phalaris tuberosa var. *hirtiglumis* Trab., *Fl. Algérie* 140 (1895); *Phalaris hirtiglumis* (Batt. & Trab.) Baldini, *Webbia* 47(1): 20 (1993).

Phalaris nodosa var. *minor* Lojac., *Fl. Sicul*. 3: 251 (1908).

Phalaris elongata Braun.-Blanq., *Bull. Soc. Hist. Nat. Afrique N*. 13: 21 (1922).

Phalaris tuberosa var. *clausonis* Maire & Trab., *Bull. Soc. Hist. Nat. Afrique N*. 24(7): 230 (1933).

Phalaris stenoptera Hack., *Repert. Spec. Nov. Regni Veg*. 5: 333 (1908); *Phalaris tuberosa* var. *stenoptera* (Hack.) Hitchc., *J. Wash. Acad. Sci*. 24(7): 292 (1934).

[*Phalaris bulbosa auctt. non, p.p*.].

Phalaris maderensis (Menezes) Menezes, *Gram. Madeira* 23 (1906)

Phalaris coerulescens var. *maderensis* Menezes, *Cat. Phanerog. Madeira, Porto Santo* 57 (1894).

Section ***Heterachne*** Dumort., *Fl. Belg.* 152 (1827)

Section ***Anomophalaris*** Tzelev., *Novosti, Sist. Vyssh. Rast.* 11: 71 (1974)

Phalaris coerulescens Desf., *Fl. Atlant.* 1: 56 (1798)

Phalaris paradoxa var. *coerulescens* (Desf.) Paunero, *Anales Jard. Bot. Madrid* 8: 486 (1948).

Phalaris bulbosa Cav., *Icon*. 1: 46, t. 64 (1791), *nom. illeg.*, *non* L. (1755).

Phalaris tuberosa Link, *J. Bot*. 4: 312 (1799), *nom illeg., non* L. (1771).

Phalaris variegata Spreng., *Neue Entdeck. Pflanzenk*. 2: 101 (1821).

Phalaris coerulescens var. *ovata* Parl., *Pl. Nov*. 33 (1842).

Phalaris coerulescens var. *tenuis* Asch. & Graebn., *Syn. Mitteleur. Fl*. 2(1): 17 (1898).

Phalaris coerulescens var. *villosula* De Not. ex Parl., *Fl. Ital*. 1: 73 (1848).

Phalaris coerulescens var. *concolor* Lojac., *Fl. Sicul*. 3: 251 (1908).

[*Phalaris aquatica auct. non* L., *p.p.*].

Phalaris paradoxa L., *Sp. Pl.*, 2nd edn, 1665 (1763)

Phalaris paradoxa var. *typica* Paunero, *Anales Jard. Bot. Madrid* 8: 486 (1948), *nom. inval.*

Phalaris praemorsa Lam., *Fl. Franç*. 3: 566 (1778); *Phalaris paradoxa* var. *praemorsa* (Lam.) Coss. & Durieu, *Expl. Sci. Algérie* 2: 25 (1854); *Phalaris paradoxa* f. *praemorsa* (Lam.) Paunero, *Anales Jard. Bot. Madrid* 8: 486 (1948).

Phalaris sibthorpii Griseb., *Spic. Fl. Rumel*. 2: 468 (1844).

Phalaris paradoxa var. *intacta* Coss. & Durieu, *Expl. Sci. Algérie* 2: 24 (1854).

Phalaris paradoxa var. *intermedia* Coss. & Durieu, *Expl. Sci. Algérie* 2: 24 (1854).

Phalaris paradoxa L. var. *megastachys* Goiran, *Nuovo Giorn. Bot. Ital., n.s.*, 17: 53 (1910).

Phalaris appendiculata Schult., *Mant.* 2: 216 (1824)

Phalaris paradoxa var. *appendiculata* (Schult.) Chiov., *Annuario Reale. Ist. Bot. Roma* 8(3): 328 (1908); *Phalaris paradoxa* f. *appendiculata* (Schult.) Chiov., *Annuario Reale. Ist. Bot. Roma* 8(3): 328 (1908).

Phalaris rubens Ehrenb. ex Trin., *Mém. Acad. Imp. Sci. Saint-Pétersbourg, Sér. 6, Sci. Math., Seconde Pt. Sci. Nat.* 5,3(3): 51 (1839).

Phalaris obvallata Trin., *Mém. Acad. Imp. Sci. Saint-Pétersbourg, Sér. 6, Sci. Math., Seconde Pt. Sci. Nat*. 5,3(3): 51 (1839).

Phalaris pseudoparadoxa Fig. & De Not., *Agrostgr. Aegypt*. 11 (1853).

Phalaris paradoxa f. *Nana* Chiov., *Annuario Reale. Ist. Bot. Roma* 8(3): 328 (1908).

Artificial hybrids used as forage and crop, not naturalised

Phalaris* × *daviesii S.T. Blake (*P. tuberosa* L. × *P. minor* Retz.), *Proc. Roy. Soc. Queensland* 67: 27 (1956) (2*n* = 8*x* = 56)

Phalaris arundinacea L. × ***P. aquatica*** L. (2*n* = 8*x* = 56)

Phalaris × *tuberinacea* Cialz., *Rev. Inv. Agric*., 16: 80 (1962), *nom. inval., nom. nud.*

Place of publication of this name is sometimes given as G.Covas and C.Cialzeta, *IDIA* 68: 8 (1953). However, although the hybrid *Phalaris arundinacea* × *P. aquatica* is discussed in that paper, the name is not published in that work. See also Covas and Cialzeta (1953) and Ferreira *et al*. (2002).

Key to the subgenera of *Phalaris*

1. Sterile lemmas chafflike, 2–3 mm long and narrow or 1/5–1/10 the length of the fertile floret and wide ..... subgenus ***Phalaris***
   Sterile lemmas hairy or tufts of hair, 1–3.5(4) mm long, if glabrous one or both sterile lemmas reduced up to be obsolete ..... subgenus ***Phalaroides***

Key to sections of subgenus *Phalaroides*

1. Sterile lemmas 1 or 2, narrow, subequal 2
   Sterile lemmas 2, equal 3
2. Sterile lemmas pubescent, 0.5–2 mm long; fertile lemmas 0.3–3 mm long ..... section ***Bulbophalaris***
   Sterile lemmas glabrous very reduced to obsolete, fertile lemmas 0.5–4 mm long ..... section ***Heterachne***
3. Sterile lemmas 2, equal, 1.5–2.5 mm long, densely feathery; fertile lemma 3–5 mm long, sparsely pubescent..... section ***Phalaroides***
   Sterile lemmas 2, equal, pubescent, sometime feathery, 1.5–2 mm long; fertile lemmas 1.5–5 mm long..... section ***Caroliniana***

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Linnaeus C (1763) 'Species plantarum 2.' (Laurentii Salvii: Holmiae, Sweden)

Linnaeus C (1767) 'Systema naturae 2.' (Laurentii Salvii: Holmiae, Sweden)

Linnaeus C (1771) 'Mantissa plantarum altera generum editionis VI et specierum editionis II.' (Laurentii Salvii: Holmiae, Sweden)

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Appendix 1. Taxa used, their geographic origin, herbarium voucher information and GenBank numbers

Species, origin, voucher, GenBank accession (ITS, *trn*T–F)
Phalaris amethystina, South America, 108407 (SGO), JF951060, JF951092
P. angusta, Louisiana, USA, R.D. Thomas 87875 (VPI), JF951112, KF753786
P. appendiculata, Ethiopia, USDA 331404, JF951071, JF951107
P. aquatica, Netherlands, USDA 284200, JF951056, JF951094
P. arundinacea, Canada, USDA 387928, JF951075, JF951095
P. brachystachys, California, USA, Lowell Ahart 10286 (CSCH), KF753780, JF951114
P. caesia, Montpellier, France, M.Kerguelen 8983 (FI), JF951061, JF951115
P. californica, California, USA, Hickman 1, KF753781, KF753789
P. canariensis, Egypt, USDA 251274, JF951058, JF951100
P. caroliniana, Georgia, USA, R.K. Godfrey 68477 (VPI), JF951079, JF951101
P. coerulescens, United Kingdom, USDA 239340, JF951066, JF951102
P. lemmonii, California, USA, D.Bramlet 2290 (RSA), JF951082, JF951117
P. lindigii, Carchi, Ecuador, P.Peterson *et al*. 3237425 (US), JF951068, JF951104
P. maderensis, Madeira Island, Portugal, J.A.Carvalho s.n. (FI), JF951083, JF951118
P. minor, South Africa, USDA 208404, JF951069, JF951105
P. paradoxa, Cyprus, USDA 239845, JF951070, JF951106
P. platensis, Argentina, USDA 281598, KF753784, KF753799
P. rotgesii, Corsica, France, R.M.Baldini 15/1 (FI), JF951074, JF951110
P. truncata, Tunisia, USDA 535561, JF951059, JF951111
Hierochloe australis, DQ631447.1
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Anthoxanthum monticola, L. J. Gillespie, A. Archambault, R. J. Soreng, unpubl. data, 2008, DQ353953.1; Kim *et al*. 2009, EF577511.1